

INSTITUTE REPORT NO. 99



THE MUTAGENIC POTENTIAL OF: 4-nitrophenyl methyl phenyl phosphinate, 4-nitrophenyl diphenyl phosphinate, 4-nitrophenyl dimethyl phosphinate; 4-chlorophenyl methyl phenyl phosphinate,

4-chlorophenyl diphenyl phosphinate

LEONARD J. SAUERS BA, SP5 FREDDICA R. PULLIAM BS, SSG JOHN T. FRUIN DVM, PhD, LTC VC AUG 201981

TOXICOLOGY SERVICES GROUP. **DIVISION OF RESEARCH SUPPORT**

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The Mutagenic Potential of 4-nitrophenyl methyl phenyl phosphinate; 4-nitrophenyl diphenyl phosphinate; 4-nitrophenyl dimethyl phosphinate; 4-chlorophenyl methyl phenyl phosphinate; 4-chlorophenyl diphenyl phosphinate was assessed using the .0000032 Ames Salmonella/Mammalian Microsome Mutagenicity Assay.

Tester Strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 were exposed to doses ranging from 0.01 mg/plate to 3.2×10^{-9} mg/plate for 4-chlorophenyl diphenyl phosphinate and 1 mg/plate to 3.2×10^{-9} mg/plate for all other test compounds. It was determined that none of the tested substances had mutagenic potential

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ABSTRACT

The mutagenic potential of:

| 4-nitrophenyl methyl phenyl phosphinate | 37 |
|--|-----|
| 4-nitrophenyl diphenyl phosphinate | 73/ |
| 4-nitrophenyl dimethyl phosphinate | 83 |
| 4-chlorophenyl methyl phenyl phosphinate | 53 |
| 4-chlorophenyl diphenyl phosphinate | 91 |

was assessed by the Ames Salmonella/Mammalian Microsome Assay.

Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 0.01 mg/plate to 3.2×10^{-6} mg/plate for 4 chlorophenyl diphenyl phosphinate and 1 mg/plate to 3.2×10^{-4} mg/plate for all other test compounds. It was determined that none of the tested substances had mutagenic potential.

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PREFACE

SUBSTANCE

| AMES | ASSAY | REPORT: | 4-nitrophenyl methyl phenyl phosphinate | 37 |
|------|-------|---------|--|-----|
| | | | 4-nitrophenyl diphenyl phosphinate | 73A |
| | | | 4-nitrophenyl dimethyl phosphinate | 83 |
| | | | 4-chlorophenyl methyl phenyl phosphinate | 53 |
| | | | 4-chlorophenyl diphenyl phosphinate | 91 |

TESTING FACILITY: Letterman Army Institute of Research Presidio of San Francisco, CA 94129

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PROJECT: Toxicity Testing of Phosphinate Compounds - 612772.875

GLP STUDY NUMBER: 80012

STUDY DIRECTOR: LTC John T. Fruin D.V.M., PhD.
CO-PRINCIPAL INVESTIGATORS: SSG Freddica R. Pulliam, B.S.
SP5 Leonard J. Sauers, B.A.

RAW DATA: A copy of the final report, study protocol and retired SOPs will be maintained in the LAIR archives. Test compounds were provided by sonsor. Chemical, analytical, stability, purity, etc. data available from sponsor.

PURPOSE: To determine the mutagenic potential of the above compounds using Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were used.

Code No.

ACKNOWLEDGMENTS

The authors wish to thank SP5 Lon Kincannon, BA; and SP5 Robert Summers for their assistance in performing the research.

Signatures of Principal Scientists Involved In The Study

we, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability, with the Good Laboratory Practice Regulations outlined by the Environmental Protection Agency.

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DEPARTMENT OF THE ARMY



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SGAD-ULZ-QA

8 January 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 80012 the following inspections were made:

28 October 1980

30 October 1980

18 November 1980

20 November 1980

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the December 1980 report to management and the Study Director.

JOHN L. SZUREK

MAJ, MS

Quality Assurance Officer

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Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tasts used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the schection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysachride layer (LP) is mutated and allows larger molecules to enter the bacteris. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rata induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated and liver microsomal enzyme homogenate is termed 5-9.

Description of Strains (History of the strains used, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of growth inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be by using ultraviolet (UV) light. determined These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California, Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data, to determine if deviations from the set trends have occurred.

We compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair

mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

METHODS (3)

Mark Commence

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. toxicity level was found by using MGA plates, various trations of the substance, and approximately 10° cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will rep'icate times and potentially express a mutation. When the biotin supplies are exhausted, only those bacteria that reverted the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution which decreased macrocolony formation, below that of the revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. the actual experiment, 0.1ml of the particular strain of Salmonella cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which just passed the log phase, the Salmonella strains were used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned more than a 1000- fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayered on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner Concentrate (4). The water used in this medium and a'l reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous

revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliablilty of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$MUTAR = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound; C_{AV} is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

RESULTS AND DISCUSSION

Throughout this report, all the test substances will be referred to by their respective code numbers:

| Substance | Code No. |
|---|----------|
| 4-nitrophenyl methyl phenyl phosphinate | e 37 |
| 4-nitrophenyl diphenyl phosphinate | 73A |
| 4-nitrophenyl dimethyl phosphinate | دَ8 |
| 4-chlorophenyl methyl phenyl phosphinat | te 53 |
| 4-chlorophenyl diphenyl phosphinate | 91 |

A series of assays was run to conclusively determine the mutagenic potential of the five substances. Data from tests that were determined to be invalid due to medium preparation errors, inadequate inoculum or control failures are not reported but are retained in the LAIR archives. On 4 Nov 80, the Ames Test was performed on 73A and 37. Due to an error in medium preparation, no

growth was present after the 48-hour incubation. This assay was repeated on 12 Nov 80. On 18 Nov 80, substances 53, 83 and 91 were tested. Throughout the assays of 12 and 18 Nov, we observed uneven lawns on plates containing test strain TA 1537. We suspected that the TA 1537 inoculum was insufficient; therefore, all five chemicals were retested on 2 Dec 80, using an inoculum of TA 1537 prepared from parent culture stock. A plating error resulted in a lack of growth on the positive control plates. The test was done again on 9 Dec 80. The spontaneous reversion level was below our historical data for nonactivated TA 98 and nonactivated TA 1538 from the 18 Nov 80 assay. The experiment was repeated on 16 Dec 80 with only TA 98 and TA 1538.

Strain verification and sterility controls were normal for all assays reported (Tables 1A - 1E). The assay of 12 Nov 80 showed a spontaneous reversion rate below that suggested by Ames et al (2) on both activated and nonactivated TA 98, TA 100, and TA 1538 also for activated TA 1535 (Table 1A). On 18 Nov 80, all the spontaneous reversion rates for the nonactivated strains were below that suggested range along with activated TA 1535 and TA 1538. Nonactivated TA 98 and TA 1538 (Table 1B) were significantly below our historical data values. The spontaneous reversion rate was low for TA 98 nonactivated on 16 Dec 80 (Table 1E). Spontaneous reversion values below that suggested by Ames et al (2) are indicative of high quality water, materials, techniques, etc. Counts higher than those suggested by Ames et al (2) are indicators of serious performance

The effects of the positive control chemicals are reported in Tables 2A - 2D. Positive control values below that expected were observed for TA 98, TA 1537 and TA 1538 to dimethyl-benzanthracene (DMBA) on 12 Nov 80 (Table 2A). On 18 Nov 80, the same results were seen for TA 98, TA 100, TA 1537 and TA 1538 (Table 2B). Below par value were also evident on 9 Dec 80 for TA 1537 to DMBA. The same was true on 16 Dec 80 for TA 98 and TA 1537 (Tabel 2D). DMBA functions as a frameshift mutagen and is used to determine if strains TA 98, TA TA 1537 and TA 1538 are functioning properly. Although the strains did not respond to DMBA, they did respond to aminofluorene (AF) and benzo()pyrene (BP), both of which are also frameshift mutagens. In all instances when n-methyl-n nitro-N-nitrosoquanidine (MNNG) was the positive conrol, test strains responded as anticipated.

The Minimum Toxicity Level Determination Assay was performed on $28\ \, 0ct\ \, 80.$ Our quality control showed that we had incurred experimental contamination on the test plates (Table 3). By observing the condition of the background lawn, the optimal sublethal dose was determined, even though extraneous growth was present. Sparse or no growth of the background lawn signified toxicity. The optimal sublethal dose was chosen at a point where a lawn having normal growth became evident (Table 4A-4E).

The data for the mutagenic potential are reported in Tables 5A - 5J. Data for test compound 37 were collected on 12 Nov 80 and 11 Dec 80. On 12 Nov 80 (Table 5A), two isolated incidences of a more than doubling of the spontaneous reversion rate occurred: activated TA 1535 at the 0.0016 mg/plate dose and activated TA 1537 at the 0.04 mg/plate level. The assay of strain TA 1537 was performed again on test substance 37 on 11 Dec 80 (Table 5G). No mutagenic activity was demonstrated. It is concluded that the response of activated TA 1537 on 12 Nov 80 for the 0.04 mg/plate dose was unexplainable and probably due to experimental error since the results could not be reproduced. The activity found in TA 1535 was disregarded due to the lack of correlation with dose response.

The data for test substance 73A were obtained on 12 Nov 80 (Table 5B) and 11 Dec 80 (Table 5G). In all occurences, no evidence of mutagenic activity was found.

Compound 83 was tested on 18 Nov 80 (Table 5C), 11 Dec 80 (Table 5G) and 16 Dec 80 (Table 5H). On 18 Nov 80, all TA 98 dose levels showed doubling or greater of the spontaneous reversion rate. This was also true for nonactivated TA 1538 at the 0.04 mg/plate dose level through the 0.00032 mg/plate dose. The spontaneous reversion rate for these nonactivated strains was below that suggested by Ames et al (2) as indicative of mutagenicity. Test substance 83 was assayed using only strains TA 98 and TA 1538 on 16 Dec 80. No mutagenic activity was presented. It was concluded that the 18 Nov 80, suggestion of mutagenic activity was due to the spontaneous reversion rate for TA 98 and TA 1538 which was far below the historical average. The MUTAR values were also insignificant.

Test substance 53 was tested on 18 Nov 80 (Table 5D), 11 Dec 80 (Table 5G), and 16 Dec 80 (Table 5I). On 18 Nov 80, a greater than twice the spontaneous reversion rate was observed for all dose levels containing nonactivated TA 98 and nonactivated TA 1538. The same occurred for nonactivated TA 1535 at the 0.008 and 0.00032 mg/plate doses. Nonactivated TA 1537 showed possible mutagenic activity at the 0.008 and 0.0016 mg/plate dose levels. On 11 Dec 80, the TA 1537 assay was repeated; no evidence of mutagenic activity was present. It was concluded that the mutagencity initially presented with TA 98, TA 1537 and TA 1538 was due to low spontaneous reversion rates. The activity found in TA 1535 was disregarded due to the low spontaneous reversion rate and the lack of correlation to dose response.

Test substance 91 was assayed on 18 Nov 80 (Table 5E), 11 Dec 80 (Table 5G) and 16 Dec 80 (Table 5J). In the assay of 18 Nov 30, a doubling or greater spontaneous reversion rate was seen for nonactivated TA 1558 and nonactivated TA 98 for all dose levels. The spontaneous reversion values were low for both of these nonactivated strains. When the assay was repeated on 11 Dec 80, no mutagenic

activity was seen. It was concluded that the initial observation of mutagencity was due the low spontaneous reversion values. All calculated MUTAR values were below the 1.5 threshold value necessary to declare a substance as a mutagen (Tables 6A-6M).

CONCLUSION

To declare that a substance is a mutagen through the Ames Test, two criteria must be met: a more than doubling of the spontaneous reversion rate and an obvious dose response. Since only a few scattered incidences of twice the spontaneous reversion rate were observed, it was concluded that compounds 37, 73A, 83, 53 and 91 are not mutagenic.

RECOMMENDATION

We recommend that organo-phosphinate compounds 37, 73A, 83, 53, and 91 be tested using other toxicological testing systems if efficacy tests show those chemicals to be promising antidotes.

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APPENDIX (Continued)

Table 1-A

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

| | | | | | 7: (: | | | | | |
|--|-----------------------------------|---------------------------------|---------------------------|------------------|-------------------------|-------------------|--------------------------|----------------------|---------------------|--------------------------|
| Strain No. | Histidi Require | | | cillin stance | | uvr-B Delet | | rfa Cr Violet | ystal (d) | Sterility Control (e) |
| TA 98 | + | | | + | | + | | 16.2 | 7 mm | NG |
| TA 100 | + | | | + | | _+ | | 16.6 | 5 mm | NG |
| TA 1535 | + | | | NA | | + | | 20.3 | 5 mm | NG |
| TA 1537 | + | , | | 26.05 | mm | + | | 18.3 | 4 mm | NG |
| TA 1538 | + | | | NA | | + | | 20.3 | O mm | NG |
| WT | grow | th | | NA | | NA | | NA | I | NA |
| | | | Δι ττν | CONTR | 201 (4 | | | | i | |
| His-Bio mix | Initi | 11: _ ^{NT} _ | | End: | NT | <i>=</i> / | T | act Comm | ound 1 | + (73A) |
| | | | | | | | | | | |
| Top Agar | | 11: | | End: | + | | | est Comp | | |
| i | | il: <u>+</u> | | End: | | | , T | est Comp | ound 3: | NA NA |
| Diluent: | + | | | Nutrie | nt Br | oth:_ | т | est Cpmp | ound 4: | tiA |
| MGA Plate w/ I | pacteria: | + | | MGA P1 | ate:_ | + | т | est Comp | ound 5: | NA |
| (a) + = no gru - = zone of in side of plate growth (growth tlA = not appli | nhibition ; (d) + h indicat | of appr zone of tes conta | oxima of inh uminat | tely labition; | 6mm; on app NT=no | (c) + roximat | ≈ no tely ì ed; NG | growth d Amm diam | n irrac meter: (| diated (e) + = no |
| Strain (1) | Avg I | Range | No | S-9 | | Avg | | S-9 | | Avg |
| TA 98 | 40 | 30-50 | 25 | 24 | 18 | 22 | 32 | 24 | 23 | 26 |
| TA 100 | 160 12 | 20-200 | 102 | 121 | 122 | 115 | 112 | 102 | 123 | 112 |
| TA 1535 | 20 | 10-35 | 14 | 16 | 8 | 13 | 6 | 3 | 5_ | 5 |
| TA 1537 | 7 | 3-15 | 14 | 11 | .8 | 11 | 15 | 4 | 10 | _10 |
| TA 1538 | 25 | 15-35 | 6 | 14 | 11 | 10 | 13 | 13 | 15_ |]4 |

Ames, B.N., J. McCann and E. Yamasaki. Mucat. Res. 31:347

| Test Inoculated By: | Summers, Sauers, Pulliam, Kinca | nggile. | 12 Nov 80 | _ |
|----------------------|---------------------------------|---------|-----------|---|
| Test Read By: Pullia | m | Date: | 14 Nov 80 | |

Table-1-B

QUALITY CONTROL OF TESTER STRAINS WORKSHEET Salmonella/Microsome Assay

| | | <u> </u> | | | | | | | |
|---------------------|---|------------------------------|--|--------------------------|------------------|-----------------------------|----------------------|------------------|--|
| Strain No. | Histidine (a) Requirements | | cillin stance | | uvr-B Delet | | rfa Cr Violet | | Sterility Control (e) |
| TA 98 | + | | + | | + | | 15.33 | men | 7G |
| TA 100 | ÷ | | + | | | | 17.22 | | NG |
| TA 1535 | | | | | | | | | NG |
| TA 1537 | + . | | 2.43 | | + | | 17.44 | mm | NG |
| TA 1538 | + | | NΑ | | + | | 20.0m | m | 11G |
| WT | Growth | | HA. | | NA. | | NA_ | <u> </u> | NT. |
| QUALITY CONTROL (e) | | | | | | | | | |
| His-Bio mix | Initial: | + | End: | + | | Te | st Comp | ound 1: | 83- NG |
| Top Agar | Initial: | + | End: | + | | Te | st Comp | ound 2: | 91- NG |
| S - 9 | Initial: | + | End: | + | | . Te | st Comp | ound 3: | 53- NG |
| Diluent: | + | | Nutrie | ent Br | oth: <u>+</u> | Te | st Cp mp | ound 4 | NA |
| MGA Plate w/ | bacteria: <u>Growt</u> | h | MGA P1 | late:_ | + | Te | st Comp | ound 5 | : <u>\\</u> A |
| - = zone of i | owth (requires inhibition of ap; (d) + = zone h indicates contable. | proxima of inh taminat | itely initional initionali | l6mm; on app NT=no | (c) + roximat | = no g ely 14 ed; NG= | prowth c Amm diam | n irra eter: | d1ated (e) + = no |
| Strain (1) | Avg Range | No | 5-9 | | Avg | | S-9 | | Avg |
| TA 98 | 40 30-50 | 4 | 0 | 3 | 2 | 29 | 41_ | 38 | 36 |
| TA 100 | 160 120-200 | 78 | 63 | 88 | 76 | 133 | 140 | 118 | 130 |
| TA 1535 | 20 10-35 | 8 | 6 | 2 | 5 | 12 | 2 | 3 | 6 |
| TA 1537 | 73-15 | *3 | 0 | 7_ | 3 | <u> </u> | 1_1_ | 8 | 5 |
| TA 1538 | 25 15- <u>35</u> | 1 | 4_ | <u> 1</u> | 1 2 | 6 | 1 13 | <u> </u> 13 | <u> </u> |
| |). McCann and E. | | | | | | | | |
| Test Inocula | ted By: <u>Sauers</u> , | Summe | rs, Pu | <u>Neill</u> | | | | | |
| Test Read By | · Pulliam | | | | | Date: | 20 № | ov 80 | |

* Sparse lawn

Table-1-C

QUALITY CONTROL OF TESTER STRAINS WORKSHEET Salmonella/Microsome Assay

| | | | | | | | | | | · |
|---|------------------|--------------------------|------------------|------------------|----------------|-------------------|------------------|----------------|-----------------|--------------------------|
| Strain No. | | dine (a) rements | | cillin stance | | uvr-b Delet | (c) ion | rfa C Viole | rystal t (d) | Sterility Control (e) |
| TA 98 | | NA | | NA NA | | | 4 | <u>N</u> A | | NA. |
| TA 100 | | NA | | NA | | N/ | A | NA | | NA |
| TA 1535 | <u> </u> | + | | MA | | N/ | 4 | <u>NA</u> | | NA . |
| TA 1537 | | + | | 18mm | | <u>+</u> | | 20m | m | 4G |
| TA 1538 | | NA | | MA | | !#/ | 1 | NA. | | NA NA |
| WT | Gro | owth | | NA | | ٧/ | 1 | <u>N</u> A | | NA NA |
| | | QL | JALITY | CONTR | <u>OL</u> (| e) | | | | |
| His-Bio mix | Init | ial: <u>+</u> | | End: | + | | T | est Com | pound 1 | : <u>!IG</u> |
| Top Agar | Init | ial: <u>+</u> | | End: | + | | T | est Com | pound 2 | : NA |
| S - 9 | Init | ial: <u>+</u> | | End: | + | | ٦. | est Com | pound 3 | 3: <u>NA</u> |
| Diluent: | TN | | | Nutrie | nt Br | oth: <u>+</u> | 1 | est Cpm | pound 4 | : NA |
| MGA Plate w/ | bacteri | a:+ | | MGA P1 | ate:_ | + | 1 | est Com | pound 5 | 5: <u>NA</u> |
| (a) + = no jr - = zone uf i side of plate growth (growt NA=not applic | ; (d) h indic | + = zone (ates conta | of int uminat | nibitio | n app NT=no | roxima: t test | tely l ed; NG | 4mm dia | meter; | (e) + = no |
| Strain (1) | Avg | Range | No | S-9 | | Avg | <u> </u> | S-9 | | Avg |
| TA 98 | 40 | 30-50 | T | | | NA | | 1 | | l na |
| TA 100 | 160 | 120-200 | | | | NA : | | | | 1A |
| TA 1535 | 20 | 10-35 | T | | | NA | | | | NA . |
| TA 1537 | 7 | 3-15 | 4 | 4 | 3 . | 4 | 7 | 8 | 2 | 6 |
| TA 1538 | 25 | 15-35 | | | | NA | | | <u> </u> | l _{NA} |
| Ames, B.N., J |). McCai | nn and E. | · Ya.mas | aki. I | Autat. | Res. | 31:34 | 7 | | |

| Test | Inoculated | By: Sauers, Summers, Pulliam | Date: | 2 Dec 80 |
|------|------------|------------------------------|-------|----------|
| Test | Read By: _ | Fullian | Date: | 4 Dec 30 |

^{*} Tested when batch first made, see data dated 18 Nov 80

Table-1-D

QUALITY CONTROL OF TESTER STRAINS WORKSHEET Salmonella/Microsome Assay

| Strain No. | Histidine (a) Requirements | | icillin istance | | uvr-l Delei | (c) tion | | rystal t (d) | Sterility , Control (2) | |
|---|--|---------------------------|---------------------------------------|-------------------------|-----------------------------|--------------------------|--------------|-----------------|----------------------------|--|
| TA 98 | <u>NA</u> | | NA | | NA NA | | N/ | | NA. | |
| TA 100 | NA NA | | NA | | NA | | N/ | 1 | NA NA | |
| TA 1535 | NA NA | | NA | | ŅΑ | | N/ | ١ | NA . | |
| TA 1537 | + | <u> </u> | 1 GMM | | + | | 1.7r | nm | NG | |
| TA 1538 | NA NA | | NA | | NA | | N/ | 1 | NA NA | |
| WT | NA NA | <u> </u> | NA | | NA | | N/ | | N3 | |
| QUALITY CONTROL (e) | | | | | | | | | | |
| His-Bio mix Initial: + End: + Test Compound 1: NG | | | | | | | | | | |
| Top Agar | Initial: <u>1 co</u> | lony | End: | + | _ | T | est Con | pound 2 | : NG | |
| S - 9 | Initial: + | | End: | + | | ر ٦ | est Con | pound 3 | : <u>NG</u> | |
| Diluent: | Diluent: + Nutrient Broth: + Test Cpmpound 4: NG | | | | | | | | | |
| MGA Plate w/ b | cacteria: <u>(WT) g</u> | <u>row</u> th | MGA P1 | ate:_ | + | T | est Com | pound 5 | : <u>NG</u> | |
| - = zone of ir | with (requires he hibition of app. (d) + = zone of indicates contable. | roxima of inl amina | ately 10 | 6mm; n appi NT=no | (c) + roximat t teste | = no tely l ed; NG | growth | on irra | diated | |
| Strain / | Avg Range |] No | S-9 | | Avg | | S- 9 | | J Avg | |
| (1) | | - | , , , , , , , , , , , , , , , , , , , | | ļ | | | 1 | - | |
| TA 98 | 40 30-50 | | | | NA : | | | | NA I | |
| TA 100 | 60 120-200 | | | | NA | <u> </u> | | | NA | |
| TA 1535 | 20 10-35 | - | <u> </u> | | NA . | | <u> </u> | ļ | NA | |
| TA 1537 | 7 3-15 | 9_ | 8 | 5 | 7 | _9_ | 8 | 6 | 3 | |
| TA 1538 | TA 1538 25 15-35 NA NA | | | | | | | | | |
| Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347 | | | | | | | | | | |
| Test Inoculated By: Pulliam, Sauers Date: 9 Dec 80 | | | | | | | | | | |
| Test Read By: | Pulliam | | | | | Date | : 11 0 | ec 80 | and the same and the same | |

Table-1-E

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

| Strain No. | Histidine (&) Requirements | | cillin (istance | (p) | uvr-8 Delet | (c) ion | rfa Cr Violet | ystal (d) | Sterility Control (e) | | |
|---|---|-------------------------|---|----------------------|---------------------------|--------------------------|--|------------------------------------|---|--|--|
| TA 98 | + | | + | | + | | 17.43 | lmm | NG | | |
| TA 100 | + | | NA | | t_ | | <u>IIA</u> | | NA | | |
| TA 1535 | NA | | NA | | NA. | | NV | | NA | | |
| TA 1537 | NA · | | NA | _ | NA | | NA | | AK | | |
| TA 1538 | + | | 23.45 | | + | | 17.21 | mm | NG | | |
| <u>kt</u> | NA NA | | NA | | Grow | .h | NA. | | AI: | | |
| | QU | ALITY | CONTROL | _ (e | e) | | | | | | |
| His-Bio mix Initial: + End: + Test Compound 1: + (53) | | | | | | | | | | | |
| Top Agar Initial: + End: + Test Compound 2: + (91) | | | | | | | | | | | |
| S - 9 | | | | | | | | | | | |
| Diluent: | Diluent: | | | | | | | | | | |
| MGA Plate w/ b | oacteria: + | | MGA Plat | te: | + | T | est Comp | ound 5 | : <u>*IA</u> | | |
| (a) + = no gro - = zone of in side of plate growth (growth NA=not applica | with (requires hinibition of appr (d) + = zone of indicates conta ble. Spo | oxima f ini minat | ine for quately 16million tion); Niceous Reve | mm; appi [≈not | (c) + roximat teste | = no cely l ed; NG | = no zon growth o 4mm diam =no grow | e of 11 n irradeter; th; WT: | enthition, diated (e) + = no wild type | | |
| Strain / | Avg Range | No | S-9 | | Avg | | S-9 | | Avg | | |
| TA 98 | 40 30-50 | 23 | 24 2 | 28 | 25 | 25 | 34 | 32 | 30 | | |
| TA '00' AT | 160 120-200 | | | | NA | | | | <u> </u> | | |
| TA 1535 | 20 10-35 | | | | 'VA | | | | NA | | |
| TA 1537 | 7 3-15 | <u> </u> | | | NA. | | | | NA | | |
| TA 1538 | 25 15-35 | 111 | 14 | 19 | 15 | 20 | 15_ | 17 | 13 | | |
| Ames, B.N., J. McCann and E. Yamasakı. Mutat. Res. 31:347 | | | | | | | | | | | |
| Test Inoculated By: Pulliam, Summers, Sauers Date: 16 Dec 80 | | | | | | | | | | | |
| Test Read By: | Sauers, Sun | mers | | | | Date | 12 | <u>Dec 80</u> | and and and | | |

TABLE 2 A

POSITIVE CONTROL REVERTANT RATE

| Date | Strain | Spontar | ieous Rev | AF | MNNG | BP | DIIBA | Re- | Init |
|--------|----------|----------|-----------|-------------|---|----------|----------|---------------|-------|
| Date | Strain | S-9 | No S-9 | s- 9 | No S-9 | S-9 | S-9 | sponse (a) | 11110 |
| 14 Nov | TA 98 | 26 | 22 | 1453 | NA | 137 | 48 | | |
| н | TA 100 | 112 | 115 | 728 | 5109 | 370 | 227 | + | |
| " | TA 1535 | 5 | 13 | NA | 16104 | NA | NA | 1 . | |
| " | TA 1537 | 10 | 11 | NA | NA | 38 | 18 | - | |
| " | TA 1538 | 14 | 10 | 1619 | NA | 77 | 18 | <u> </u> | |
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(a) + = expected result, - = unexpected result (see discipling note) TA 98, TA 1537, and TA 1538 showed an unexpected low response to DMBA.

TABLE 2-B
POSITIVE CONTROL REVERTANT RATE

| Date | Stz · in | Spontar | eous Rev | AF | MNNG | BP | DEBA | Re- | Init |
|--|--|--|--|--|--|---|--------------|----------------|--|
| Date | 201 | 2-9 | No S-9 | s - 9 | No 5-9 | S-9 | S-9 | sponse (a) | |
| 18 Nov | TA 98 | 36 | 2 | 851 | NA | 83 | 43 | | |
| # | TA 100 | 130 | 76 | 475 | 1955 | 161 | 1117_ | | <u> </u> |
| 11 | TA 1535 | 6 | 5 | NA | 1392 | NA | NA | + | |
| 11 | TA 1537 | 5 | 3 | NA | NA. | 28 | 9 | <u> </u> | |
| # | TA 1538 | 11 | 2 | 980 | NA_ | 26 | 18 | <u> </u> | <u> </u> |
| | 1 | <u> </u> | | | | | | | <u> </u> |
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(a) += expected result, -= unexpected result (see discipline note)
TA 93, TA100, TA 1537, and TA 1538 showed an unexpected low response
to DMBA.

TABLE 2-C
POSITIVE CONTROL REVERTANT RATE

| Date | Strain | Spontan | eous Rev | AF | MNNG | BP | DUBA | Re- | Init |
|--------------|--------------|--------------|--------------|-----------|--|----------|----------------|----------------|------|
| Mte | Strain | S-9 | No S-9 | s-9 | No S-9 | S-9 | S-9 | sponse | |
| 4 Dec | TA 1537 | 6 | 4 | NG | NA | #IG | NG | - | |
| | | | | | | | | | |
| 9 Dec | TA 1537 | 8 | 7 | 14 | NA | 26 | 13 | - | |
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| } - | | | | | | | - | | |
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| | | - | | | | | - | - | - |
| | | - | | | | | | - | - |
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| - | | | | | | <u> </u> | | | |
| | | 1 | 1 | | <u> </u> | <u> </u> | | 1 | 1 |

(a) + = expected result, - = unexpected result (see discipline note)

Plating error caused lack of growth in assay of 4 Dec. TA 1537 showed an unexpected low response to DHBA in assay of 9 Dec.

TABLE 2-D
POSITIVE CONTROL REVERTANT RATE

| | Strain | Spontan | eous Rev | AF | MNNG | BP | DUBA | Re- | Init |
|----------|--|--|--------------|--|--|-------------|--|---|------------|
| Date | Strain | s-9 | No S-9 | S-9 | No S-9 | S- 9 | s- 9 | sponse (a) | 11.10 |
| 16 Dec | TA 98 | 30 | 25 | 1055 | NA. | 59 | 26 | | |
| 11 | TA 1538 | 18 | 15 | 984 | NA. | 50 | 28 | | |
| | <u> </u> | | | | | | | | |
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(a) - = expected result, - = unexpected result (see discipline note)

TA 98 and TA 1538 showed an unexpected low response to DMBA. TA 98 showed an unexpected low response to BP.

Table-3

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

| Strain No. | Histidine (a) Requirements | Ampicillian (b) Resistance | uvr=B (c) Deletion | rfa Crystal Violet (d) | Sterility Control (e | | | | | | |
|--|-------------------------------|-------------------------------|-----------------------|---------------------------|-------------------------|--|--|--|--|--|--|
| TA 100 | + | + | + | 15mm | NG | | | | | | |
| TA 1537 | NT | 24 mm | NT | NT | NG | | | | | | |
| WT | Growth | NT | Growth | NT | NT | | | | | | |
| Diluent | NT | NT | NT | NT | NG | | | | | | |
| Test Compound (s | 5) | | | | 3 | | | | | | |
| #1 | NT | NT | NT | ти | nt | | | | | | |
| #2 | NT | NT | NT | NT | 7 | | | | | | |
| #3 | NT | NT | NT | NT | nue. | | | | | | |
| #4 | • | NT | NT | NT | 1 | | | | | | |
| # 5 | NT | NT | NT | NT | 6. | | | | | | |
| (a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irratiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; WT= wild type. Spontaneous Revertants | | | | | | | | | | | |
| Strain | Average Ra | inge | | | Average | | | | | | |
| | | | | un aton | | | | | | | |

| Test Inoculate | d By: <u>Sauers, Summers, Kellner</u> | Date: <u>28 Oct 90</u> |
|----------------|---------------------------------------|------------------------|
| Test Read By: | Pulliam | Date: 31 Oct 90 |

Table 4-A TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

| Substance assayeu: | · | | | | |
|--------------------------------|--|---------------------|--|--------------------|---|
| (3) | (4) | | (5 |) | |
| Date: | Perfor | rmed by: <u>s</u> a | wers. Kinc | nnon, Pulliam | Summers |
| Substance dissolved | in: (1) <u>DMS</u> | (2) | | (3) | |
| (4)(5) | | | l estimatio ent Agar Pl TA 100 nt Plate Co | ST = s1 NL = no | d lawn on growth ight growth rmal growth |
| Test Compound Concentration | Plate #1 | | | Average | Background Lawn |
| 1.0 mg/plate | | | | | NL |
| 0.1 mg/plate | - | XC | | | NL |
| 0.01 mg/plate | | | ROX OF THE PARTY O | | NI |
| 0.001 mg/plate | | | Arrest. | | и |
| 0.000.1 mg/plate | <u> </u> | | | | NL NL |
| 0.000.01 mg/plate | | | | | NI |
| 0.000,001 mg/plate | | | | | NI. |
| 0.000,000,1_mg/plate | | | | | NI. |
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Table 4-B

TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

| Substance assayed: | (1) <u>Code #</u> | 73 | (2) | | | | | |
|--------------------------------|--------------------|--|--------------------------------------|---|---|--|--|--|
| (3) | (4) | | (5 |) | | | | |
| Date: | Perfo | med by: <u>Sauers, Kincannon, Pulliam, Summers</u> | | | | | | |
| Substance dissolved | in: (1) <u>DMS</u> | <u>o</u> (2) | | (3) | | | | |
| (4)(5) | | Visua Nutri | l estimatio ent Agar Pl TA 100 | n of backgroun ates: NG = no ST = s1 NL = no | d lawn on growth ight growth rmal growth | | | |
| Test Compound Concentration | Plate #1 | Reverta | nt Plate Co | | Background Lawn | | | |
| 1.0 mg/plate | | | | | ST | | | |
| 0-1 mg/plate | | | | | NL | | | |
| 0.01_mg/plate | | \rightarrow | | | NL | | | |
| u.001_mg/plate | | | Lever | | NI. | | | |
| 0.000.1_mg/plate | | | Son Con Rect | | - NT | | | |
| 0.000.01 mg/plate | ļ | | | | NL | | | |
| 0.000.001 mg/plate | | | | | NI | | | |
| 0.000,000,1 mg/place | | | | | NL | | | |
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Table 4-C
TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

| Substance assayed: | (1) <u>Code #</u> | 83 | (2) | | |
|------------------------|---|-------------------|-----------------------|--|---|
| (3) | (4) | | (5 |) | |
| Date: <u>28 Oct 80</u> | Perfor | med by: <u>sa</u> | uers. Kinca | innon, Pullian, | Summers |
| Substance dissolved | in: (1) <u>DMS</u> | <u>o</u> (2) | | (3) | |
| (4)(5) | *************************************** | Visua Nutri | ent Agar Pl TA 100 | NL = nor | lawn on growth ght growth mal growth |
| Test Compound | | | nt Plate Co | | Background |
| Concentration | Plate #1 | Plate #2 | Plate #3 | Average | Lawn |
| 1.0 mg/plate | | | | | NL |
| 0.1 mg/plate | | | | | NL NL |
| 0.01 mg/place | | S | | | NI. |
| 0.001 mg/plate | | ,,, | وكو | | NI. |
| 0.000.1 mg/plate | | | - Grand | | NI. |
| 0.000.01 mg/plate | ļ | | X port by the XX | u . | NL. |
| 0.000,001 mg/plate | ļ | | | | NI. |
| 0.000,000,1 mg/plate | | | | | NL |
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* Table 4-D TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

| Substance assayed: | (I) Code | #53 | (2) . | | |
|--|--------------------|--------------------|--------------------------------------|----------------|--|
| (3) | (4) | | (5 |) | ~ |
| Date: | Perfor | med by: <u>s</u> a | uers. Kinca | nnon, Pulliam | . Summers |
| Substance dissolved | in: (1) <u>DMS</u> | <u>o(2)</u> | | (3) | |
| (4)(5) | - | | l estimatio ent Agar Pl FA 100 | ST = sl | nd lawn on growth light growth ormal growth |
| Test Compound Concentration | Plate #1 | | nt Plate Co Plate #3 | unt Average | Background Lawn |
| O mg/plate | | | | | NL NL |
| .1 mg/plate | | | | | NT NT |
| .01 mg/plate | | 74 | X R. A. A. A. A. | | NL NL |
| .000.1 mg/plate | | | * Parker | K | NI. |
| .000.01 mg/plate | | | | 2 | NI. |
| .000,001 mg/plate .000,000,1 mg/place | 1 | | | | NI. |
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Table 4-E TOXICITY LEVEL DETERMINATION Salmonella/Microsome Assay

| Substance assayed: | (i) Code | #91 | (2) | | |
|--------------------------------|---|--------------------|--------------------------------------|--|--|
| (3) | (4) | | (5 |) | |
| Date:28_Oct_80 | Perfor | rmed by: <u>Sa</u> | wers. Kinc | innon, Pullia | m. Summers |
| Substance dissolved | | | | (3) | |
| (4)(5) | *************************************** | Visua Nutri | l estimatio ent Agar Pl TA 100 | n of backgro ates: NG = : ST = NL = | und lawn on no growth slight growth normal growth |
| | | | nt Plate Co | unt | |
| Test Compound Concentration | Plate #1 | | | Average | Background Lawn |
| 1.0 mg/plate | | | | | NG |
| 0.1 mg/plate | | | | | st |
| 0.01 mg/plate | | S | | | NL |
| 0.001 mg/plate | | 7 | 6 | | NL |
| 0.000.1 ing/place | <u> </u> | | X and XX | | NL |
| 0.000,01 mg/plate | <u> </u> | | | 2 | NL |
| 0.000,001 mg/plate | <u> </u> | | | | NI. |
| 0.000,000,1 mg/plate | ļ | | | | NI. |
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Table-5-A
SALMONFLLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

| | Substance Assa | yed: (| 1) | Code | #37 | (| 2) | | | | |
|-----|---|--------|-------------------------|------|----------|---------|-------|------|-------------|---------|-------|
| | (3) | | (4 |) | | | (5) _ | ÷ ., | | | |
| | Date: 12 Nov 80 Performed By: Sauers, Pulliam, Kincar | | | | | | | | | Summers | |
| | Substance diss | o1 ved | in: (1) <u>DMSO</u> (2) | | | | | | | | |
| | (3) | | (4)(5) | | | | | | | | |
| | | 1 | • | # R | levertan | t/Plate | _ | | | | |
| Sub | Conc | 98 | 98A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 1538A |
| 37 | 1.0 mg/p1 | 12 | 13 | 60 | 66 | 6 | 5 | 6 | 7 | 5 | 7 |
| 37 | 0.2 mg/p1 | 12 | 19 | 83 | 92 | 10 . | 4 | 5 | 7 | 10 | 21 |
| 37 | 0.04 mg/pl | 14 | 28 | 89 | 97 | 6 | 7 | 9 | 110 | 10 | 25 |
| 37 | 0.008 mg/p1 | 21 | 23 | 75 | 88 | 11 | 6 | 8 | 8 | 9 | 17 |
| 37 | 0.0016 mg/p1 | 18 | 22 | 84 | 77 | 10 | 12 | 7 | 12 | 12 | 14 |
| 37 | 0.00032 mg/pl | 18 | 19 | 112 | 89 | 12 | 6 | 6 | 7 | 5 | 13 |
| | Spon. Rev. | 22 | 26 | 115 | 112 | 13 | 5 | 11 | 10 | 10 | 14 |
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Table-5-B

SALMONELL A/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

| | Substance Assa | yed: | (1) | Code | #73A | (| (2) | | | | |
|----------|---|----------|----------|--------|---------|-----------------|--|------|-------|------|-------|
| | (3) | (4 |) | | | (5) | | | | | |
| | Date: 12 Nov | 80 | | _ Perf | ormed B | ly: <u>Kinc</u> | y: <u>Kincannon, Summers, Sauers, Pulli</u> am | | | | |
| | Substance dissolved in: (1) <u>DMSO</u> | | | | | (2) | | | | | |
| | (3) | | (| 4) | | | (5) | | | | |
| | | • | • | # R | evertar | t/Plate | <u>_</u> | | | | |
| Sub | Conc | 98 | 98A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 15384 |
| 73A | 1.0 mg/p1 | 9 | 14 | 47 | 54 | 9 | 55 | 44 | 2 | 8 | 15 |
| 73A | 0.2 mg/p1 | 14 | 21 | 63 | 68 | 8 | 7 | _5 | _6_ | _10_ | 14_ |
| 73A | 0.04 mg/pl | 16 | 21 | 80 | 90 | 10_ | | 8 | 6 | 8 | 22 |
| 73A | 0.008 mg/pl | 14 | 16 | 65 | 90 | 7 | 8 | 10 | 6 | _6_ | 12_ |
| 73A | 0.0016 mg/pl | 12 | 24 | 74 | 95 | 7 | 8 | | 9 | 10 | 14_ |
| 73A | 0.00032 mg/p1 | 13 | 15 | 76 | 80 | 5 | 9 | 5 | 1_2 | 8 | 14_ |
| | | | ļ | ļ | | | | | | | |
| ļ | Spon. Rev. | 22 | 26 | 115_ | 112 | 13 | 5 | | 10 | 10 | 14 |
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Table-5-C SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

| | Substance Assa; | yea: | (1) | Jue #0. | , | ١ ــــــ ١ | .41 | | | | |
|-----|-----------------|-------|-------|-------------|--------------|--|-----------|--------------|-------------|----------------|-------|
| | (3) | | (4 |) | | | (5) _ | | | | |
| | Date: 18 Nov 8 | 30 | | Perf | ormed B | y: <u>Kin</u> | cannon, S | ummers, | Sauers, | <u>Pull</u> ia | m |
| | Substance diss | olved | in: (| 1) <u>D</u> | 150 | ······································ | (2) | | | | |
| | (3) | | (| 4) | | | (5) | | <u> </u> | | |
| | | 1 | ٠ | # R | evertan | t/Plate | <u>.</u> | | | | |
| Sub | Conc | 98 | 98A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 1538A |
| 83 | 1.0 mg/p1 | 11 | 10 | 47 | 40 | 4 | 6 | 1 | 1 | 4 | 7 |
| 83 | 0.2 mg/pl | 19 | 22 | 81 | 71 | 9 | 7 | 3 | 4 | 3 | 12 |
| 83 | 0.04 mg/pl | 17 | 19 | 84 | 96 | 5 | 7 | 5 | 3 | 6 | 12 |
| 83 | 0.008 mg/pl | 19 | 22 | 85 | 84 | 6 | 6 | 3 | 3 | 7 | 15 |
| 83 | 0.0016 mg/p1 | 20 | 17 | 66 | 60 | 6 | 4 | 1 | 4 | 13 | 7 |
| 83 | 0.00032 mg/p1 | 12 | 15 | 51 | 83 | 6 | 7 | 3 | 2 | 6 | 11 |
| | | | | | | | | • | | | |
| | Spon. rev. | 2 | 36 | 76 | 130 | 5 | 6 | 3 | 5 | 2 | 11 |
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Table-5-D SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

| | Substance Assa | yed: (| (1) <u>Co</u> | de #53 | | (| 2) | | | | |
|------|----------------|--------|---------------|---------|---------------|---------|--|-------------|---------|------|-------------|
| | (3) | | (4 | ·) | | | 1535 1535A 1537 1537A 1538 1 6 4 3 2 14 8 7 4 3 9 9 9 2 5 5 12 7 6 5 11 6 9 9 6 6 9 13 8 3 6 9 | | | | |
| | | | | | | | | | | | |
| | Substance diss | olved | in: (| 1) | DHSO | | (2) | | | | |
| | (3) | | (| (4) | , | | (5) | | <u></u> | | |
| | | • | ٠ | # 18 | evertar | t/Plate | | | | | |
| Sub | Conc | 98 | 98A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 1538A |
| 53 | 1.0 mg/p1 | 17 | 25 | 66 | 74 | 6 | 44 | 3 | 2 | 14 | 13 |
| 53 | 0.2 mg.pl | 15 | 20 | 89 | 86 | 88 | 1 | 4 | 3 | | |
| 53 | 0.04 mg/pl | 17 | 18 | 75 | 85 | 9 | 9 | 2 | 5 | 5 | 1L_ |
| 53 | 0.008mg/p1 | 17 | 23 | 84 | 93 | 12 | 7 | 6 | 5 | _11_ | 16 |
| 53 | 0.0016 mg/p1 | 16 | 25 | 87 | 82 | 6 | 9 | 9 | 6 | 6 | 16 |
| 53 | 0.00032 mg/p1 | 13 | 25 | 70 | 90 | 13 | 8 | 3 | 6 | 9 | u_ |
| | | | | | <u> </u> | | | | | | |
| ~~~~ | Spon. Rev. | 2 | 36 | 76 | 130 | 5 | 6 | 3 | 5 | 2 | |
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Table-5-E
SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

| | Substance Assay | /ed: (| (1) <u>Co</u> | de #91 | | (| 2) | | | | |
|-----|-----------------|--------|---------------|--------|---------|-----------------|-----------|---------|---------|-----------------|-------|
| | (3) | · | (4 |) | | | (5) _ | - ,, | | | |
| | Date: 18 Nov 8 | 0 | | Perf | ormed E | By: <u>Kinc</u> | annon, Si | ummers, | Sauers. | <u>Pull</u> ian | 1 |
| | Substance disso | olved | in: (| 1)[| MSO | | (2) | | | ~~~ | |
| | (3) | | (| 4) | | | (5) | | | | |
| | | • | • | # R | evertar | t/Plate | 1 | | | | |
| Sub | Conc | 98 | 98A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 1538A |
| 91 | 0.01 mg/pl | 19 | 21 | 73 | 77 | 9 | 9 | 33 | 4 | 8 | 10 |
| 91 | 0.002 mg/pl | 20 | 21 | 66 | 69 | 6 | 7 | 4 | 3 | -6 | 9 |
| 91 | 0.0004 mg/pl | 16 | 23 | 72 | 75 | 9_ | 7 | 6 | 4 | 6 | 9 |
| 91 | 0.00008 mg/p1 | 15 | 24 | 36 | 76 | 6 | 10 | 3 | 6 | 6 | 11 |
| 91 | 0.0000032mg/p1 | 22 | 19 | 34 | 77 | 10 | 9 | 4 | , | 9 | 15 |
| | | | | | | | | | | | |
| | Spon. Rev. | 2 | 36 | 76 | 130 | 5 | 6 | 3 | 5 | 2 | 11 |
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Table-5-F SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

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| | Substance Assay |)Code #37 | | | (| (2) <u>Code #73A</u> | | | | | |
|-------|--------------------|-----------|-------|------|----------|----------------------|--------|-------------------|------------|--------|------------------------|
| | (3) Code #83 | | (4) | Co | de #53 | | (5) _ | Code | 491 | | |
| | Date: 4 Dec 8 | 0 | | Perf | ormed By | :_Kinc | annon, | Summ e rs, | Sauers, | Pullia | m |
| | Substance disso | olved in: | (1) | | DMSO | | | | | | |
| | (3) | | _ (4) | | ······ | | (5) | | | | |
| | | • | • | # Re | evertant | /Plate | - | | | | |
| Sub | Conc | 98 9 | 8A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 1538A |
| 37 | 1.0 mg/pl | | | | | | | Toxic | Toxic | | |
| 37 | 0.2 mg/p1 | | | | | | | 1 | 1 | | |
| 37 | 0.04 mg/pl | | | | | | | 3 | 3 | | -t-C-reter-Ton-science |
| 37 | 0.008 mg/pl | | | | | | | 2 | 3 | | |
| 37 | 0.0016 mg/pl | | | | | | | 3 | 3 | | |
| 37 | 0.00032 mg/pl | | , | , | | | | 4 | 2 | | |
| | | | | | | | | | | | |
| 73A | 1.0 mg/pl | | | | | | | Toxic | Toxic | | |
| 73A | 0.2 mg/pl | | | | | | | 2 | 2 | | |
| 73A | 0.04 mg/p1 | | | | | | | 4 | 4 | | |
| 73A | 0.008 mg/pl | | | | | | | 3 | 2 | | |
| 73A 0 | 0016 mg/p1 | | | | | | | 2 | 3 | | |
| 73A | 0.00032 mg/pl | | | | | | | 4 | 3 | | |
| | | | | | | | | | | | |
| 83 | 1.0 mg/pl | | | | | | | 3 | 1 | | |
| 83 | 0.2 mg/pl | | | | | | | 3 | 3 | | |
| 83 | 0 .04 mg/pl | | | | | | | 4 | 3 | | |
| 83 | 0.008 mg/p1 | | | | | | | 4 | 5 | | |
| 83 | 0.0016 mg/pl | | | | | | | 5 | 2 | | |
| 83 | 0.00032 mg/p1 | | | | | | | 5 | 3 | | |
| | | | | | | | | | | : - | ~ |

SALMONELLA/ MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND) Table-5-F Continuation Page

Revertant/Plate

| Sub | Conc | 98 | 98A_ | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 15::: |
|------|--|----|------|-----|-------------------|------|-------|----------|---------------------------------------|------|-------|
| 53 | 1.0 mg/p1 | | | | | | | 4 | 2 | | _ |
| 53 | 0.02 ing/pl | | | | ! | | | | 3 | | |
| _53_ | 0.04 mg/p: | | | | | | | 4 | 3 | | |
| 53 | 0.003 mg/;1 | | | | | | | 2 | 2 | | |
| 53 | 0.0016 mg/p1 | | | | | | | 2 | 3 | | |
| 53 | 0.00032 mg/pl | | | | | | | 5 | 3 | | |
| 91 | 0.01 ing/p1 | | | | | | | 3 | 2 | | |
| 91 | 0.002 mg/p1 | | | | | | | 3 | 2 | | |
| 91 | 0.0004 mg/pl | | | | | | | 4 | 3 | | |
| 91 | 0.00008 mg/pl | | | | - | | | 2 | 2 | | |
| 91 | 0.000016mg/p1 | | | | | | | 2 | 2 | | |
| 91 | 0.0000032mg/p | | | | | | | 2 | 2 | | |
| ,. | Spon. Re/ | | | | | | | 4 | 6 | | |
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Table-5-G SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

| | Substance Assa | yed: (| (1) _6 | ode #3 | | (| 2) | Code # | 73A | | |
|------|---------------------|--------|--------|------------|---------|----------|----------|----------|---------|---------|-------|
| | (3) <u>Code #83</u> | | (4 |) <u> </u> | ode #53 | | (5) _ | Code #9 | 1 | | |
| | Date: 11 Dec | 80 | * | _ Perf | ormed B | y: Sauer | rs, Summ | ers, Kir | cannon, | Pulliam |) |
| | Substance diss | olved | in: (| 1) | Ditiso | | (2) | | | | |
| | (3) | | | | | | | | - | | |
| | 111 | , | , | | evertan | | | | | | |
| Sub | Conc | 98 | ARP | | 100A | | | 1537 | 1537A | 1538 | 15384 |
| 37 | 1.0 mg/p1 | | | | | | | 3 | 6 | _, | |
| 37 | 0.2 mg/p1 | | | | | | | 4 | 4 | | |
| 37 | 0.04 mg/pl | | | | | | | 5 | 9 | | |
| 37 | 0.008 mg/pl | | | | | | | 4 | 7 | | |
| 37 | 0.0016 mg/pl | | | | | | | 7 | 7 | | |
| 37 | 0.00032 mg/pl | | | | | | | 4 | 4 | | _ |
| | | | | | | | | | | | |
| 73A | 1.0 mg/pl | | | | | | | 7 | 6 | | |
| 73A | 0.2 mg/pl | | | | | | | b | 5 | | |
| 73A | 0.04 mg/pl | | | | | | | 4 | 6 | | |
| 73A | 0.008 mg/p1 | | | | | | | 4 | 9 | | |
| 73A | 0.0016 mg/p1 | | | | | | | 6 | 7 | | |
| 73A | 0.00032 mg/p1 | | | | | | | 4 | 7 | | |
| | | | | | | | | | | | |
| 83 | 1.0 mg/pl | | | | | | | 6 | 4 | | |
| 83 | 0.2 mg/pl | | | | | | | 44 | 6 | | |
| 83 | 0.04 mg/p1 | | | | | | | 4 | 7 | | |
| . 83 | 0.008 mg/p1 | | | | | | | 7 | _8 | | |
| 83 | 0.0016 mg/p1 | | | | | | | 5 | _5 | | |
| _83_ | 0.00032 mg/p1 | | | | | | | _1 | 7 | | |
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SAI MONFLLA/ MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND) Table-5-G Continuation Page

Revertant/Plate

| Sub | Lonc | 98 | 98A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 1538A |
|-----|---------------|----|-----|-----|------|------|-------|------|-------|------|-------|
| 53 | 1.0 mg/pl | | | ļ | | | | 6 | 6 | | |
| 53_ | 0.2 mg/p1 | | | | | | | 5 | 7 | | |
| 53 | 0.04 mg/pl | | | | | | | 4 | 7 | | |
| 53 | 1c\pm 800.0 | | | | , | | | 4 | _10 | | |
| 53 | 0.0016 mg/pl | | | | | | | 4 | 7 | | |
| 53 | 0.00032 mg/p1 | | | | | | | 6 | 6 | | |
| | | | | | | | | | - | | |
| 91 | 0.01 mg/p1 | | | | | | | 5 | 7 | | |
| 91 | 0.002 mg/p1 | | | | | | | 6 | 9 | | |
| 91 | 0.0004 mg/p1 | | | | | | | 5 | 6 | | |
| 91 | 0.00008 mg/p1 | | | | | | | 7 | 8 | | |
| 91 | 0.000016mg/p1 | | | | | | | 7 | 6 | | |
| 91 | 0.0000032ng/p | | | | | | | 5 | 7 | | |
| | | | | | | | | | | | |
| - | Spon. Rev. | | | | | | | 7 | 8 | | |
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Table-5-H SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

| | Substance Assa | yed: | (1) | ode # 8 | 33 | (| 2) | | | ~ | |
|-------------|----------------|--|-----|---------|---------|----------------|-----------|-------------|-------------|-------|--|
| | (3) | | (4 | .) | | | (5) | | | | |
| | Date: 16 Dec | 80 | | _ Perf | ormed B | y: <u>Pull</u> | iam, Summ | ers, Sa | uers, Ke | llner | |
| | | | | | , | | | | | | |
| | Substance diss | | | | | | (2) | | | | |
| | (3) | | (| 4) | | | (5) | | | | |
| | | 1 | • | # R | evertan | t/Plate | <u>.</u> | | | | |
| Sub | Conc | 98 | 98A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 1538 |
| 83 | 1.0 mg/p1 | 9 | 16 | | | | | | _ | 12 | |
| 83 | 0.2 mg/pl | $ _{\mathbf{u}}$ | 17 | | | | | | | 11 | |
| 83 | 0.04 mg/pl | 16 | 19 | | | | | | | 14 | |
| 83 | 0.008 mg/pl | 14 | 21 | | | | | | | -8 | |
| 83 | 0.0016 mg/p1 | 17 | 20 | | | | | | | 6 | |
| 83 | 0.00032 mg/p1 | 17 | 16 | | | | | | | 6 | |
| | | | | | | | | | | | |
| | Spon Rev. | 25 | 30 | | | | | | | 15 | |
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Table-5-I SALMONEI.LA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

| | Substance Assa | yed: | 1: (1) <u>Code #53</u> (2) | | | | | | | | |
|---------------|----------------|-------|----------------------------|--------------|----------|------------------|----------|----------|-------------|--------|-------|
| | (3) | | (4 | | | | (5) _ | ٠, | | | |
| | Date: 16 Dec | 80 | | _ Perf | ormed B | y: <u>Pull</u> : | iam. Kel | lner. Sa | uers. S | ummers | |
| | Substance diss | olved | in: (| 1) <u>DM</u> | 50 | | (2) | | | | |
| | (3) | | (| 4) | | | (5) | | · | | |
| | | , | • | # R | levertan | t/Plate | | | | | |
| Sub | Conc | 98 | 98A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 1538/ |
| 53 | 1.0 mg/p1 | 14 | 18 | | | | | | | 9 | |
| 53 | 0.2 mg/p1 | 16 | 17 | | | | | | | 14 | |
| 53 | 0.04 mg/p1 | 19 | 17 | | | | | | | _10 | |
| 53 | 0.008 mg/p1 | 15 | 10 | | | | | | | 77 | |
| 53 | 0.0016 mg/rl | 17 | 20 | | | | | | | 9 | |
| 53 | 0.00032 mg/pl | 11 | 8 | | | | | | | 7 | |
| - | | | | | | | | | | | |
| | Spon. Rev. | 25 | 30 | | | | | | | 15 | |
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Table-5-J SALMONELLA/MICROSOME ASSAY WORKSHEET (POSITIVE CONTROLS/TEST COMPOUND)

| | Substance Assay | /ed: (| (1) <u>Co</u> | de #91 | | (| 2) | | | | |
|-----|-----------------|--------|---------------|---------------|---------|----------------|-----------|---------------|-------------|-------|-------|
| | (3) | | (4 |) | | | (5) | - | | | |
| | Date: 16 Dec | 80 | | _ Perf | ormed B | y: <u>Pull</u> | iam. Sauc | rs. Sum | mers. Ke | llner | |
| | Substance disso | olved | in: (| 1) <u>DMS</u> | 50 | ··· | (2) | | | | |
| | (3) | | (| 4) | | | (5) | | | | |
| | | 1 | • | # R | evertan | t/Plate | <u>.</u> | | | | |
| Sub | Conc | 98 | 98A | 100 | 100A | 1535 | 1535A | 1537 | 1537A | 1538 | 1538A |
| 91 | 0.01 mg/p1 | 8 | 19 | | | | | | | 6 | |
| 91 | 0.002 mg/p1 | 14 | 22 | | | | | | | 10 | |
| 91 | 0.0004 mg/p1 | 11 | 18 | | | | | | | _12 | |
| 91 | 0.00008 mg/pl | 13 | 19 | | | | | | | 10 | |
| 91 | 0.000016mg/p1 | 14 | 23 | | | | | | | 14 | |
| 91 | 0.0000032mg/pl | 13 | 14 | <u> </u> | | | | | | 3 | |
| | | | | | | | | | | | · |
| | Spon. Rev. | 25 | 30 | | | | | | | 15 | |
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Table-6-A

Salmonella/Microsome Assay

DMSO

| Subs | tai | nce As | ssayed: | Code | #37 | | | Dissolv | ed In: | DMSO | | |
|---------------------------------------|------|-----------|---------|----------|--------|-------|------------|------------|-------------|------------------------|--------------|-------------|
| Date | e: . | 12 No | 08 v | Per | formed | By: _ | Pulliam | , Sauers | | | | |
| Test Compound and Concentration | | TA (a) | (b) | E act | (c) | C | E-C (d) | E-C act | CAV (e) | C _{AV} act | HUTAR (f) | MUTA act |
| 1.0 mg/pl | TA | 98 | 12 | 13 | 22 | 26 | | | | | | |
| 0.2 mg/pl | TA | 98 | 12 | 19 | 22 | 26 | | | | | | |
| 0.04 mg/pl | TA | 98 | 14 | 28 | 22 | 26 | | | ** | | | |
| 0.008 mg/pl | TA | 98 | 21 | 23 | 22 | 26 | | | | | | |
| 0.0016 mg/p1 | TA | 98 | 18 | 22 | 22 | 26 | | | | | | |
| 0.00032 mg/pl | TA | 98 | 18 | 19 | 22 | 26 | | | | | | |
| 1.0 mg/p1 | TA | 100 | 60 | 66 | 115 | 112 | | | | | | |
| 0.2 mg/pl | TA | 100 | 83 | 92 | 115 | 112 | | | | | | |
| 0.04 mg/p1 | TA | 100 | 89 | 97 | 115 | 112 | | | | | | |
| 0.003 mg/p1 | TA | 100 | 75 | 88 | 115 | 112 | | | | | | |
| 0.0016 mg/pl | TA | 100 | 84 | 77 | 115 | 112 | | | | | | |
| 0.00032 mg/p1 | TA | 100 | 112 | 89 | 115 | 112 | | | | | | |
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(a)= tester strain: (b)=no. of experimental revertant colony forming units. (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$: act = activation with S-9

Table-6-A

Salmonella/Microsome Assay

| Sub | stance As | sayed: | Cod | e #37 | | | _ Dissolv | ed In: _ | DMSO | | |
|---------------------------------------|----------------|----------|----------|--------------|-------|------------|----------------|-------------------------|------------------------|--------------|--------------|
| Dat | e: <u>12 N</u> | ov 80 | Per | formed | By: _ | Pulliar | n, Sauers | | | | |
| Test Compound and Concentration | (a) | E (b) | E act | (c) | C | E-C (d) | E-C act | C _A y (e) | C _{Ay} act | HUTAR (f) | MUTA- act |
| 1.0 mg/pl | TA 1535 | 6 | 5 | 13 | 5 | | | | | | |
| 0.2 mg/pl | TA 1535 | 10 | 4 | 13 | 5 | | | | | | |
| 0.0004 mg/pl | TA 1535 | 6 | 7 | 13 | 5 | | 2 | * | 13.3 | | 0.15 |
| 0.008 mg/p1 | TA 1535 | 11 | 6 | 13 | 5 | | 1 | | 13.3 | | 0.8 |
| 0.0016 mg/p1 | TA 1535 | 10 | 12 | 13 | 5 | | 7 | | 13.3 | | 0.53 |
| 0.00032 mg/p1 | TA 1535 | 12 | 6 | 13 | 5 | | 1 | | 13.3 | | 0.8 |
| 1.0 mg/pl 0.2 mg/pl | TA 1537 | 6 | 7 | 11 | 10 | | | | | | |
| 0.04 mg/pl | TA 1537 | 9 | 110 | 11 | 10 | | 100 | | 7.5 | | 13.3 |
| 0.008 mg/pl | TA 1537 | 8 | 8 | 11 | 10 | | | | ļ | | |
| 0.0016 mg/pl | TA 1537 | 7 | 12 | 11 | 10 | | 2 | | 7.5 | | 0.27 |
| 0.00032 mg/p1 | TA 1537 | 6 | 7 | 11 | 10 | <u> </u> | ļ | | ļ | | |
| | | ļ | ļ | | | ļ | | | <u> </u> | | |
| | <u> </u> | <u> </u> | ļ | ļ | ļ | | <u> </u> | ļ | | | |
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(a)=tester strain: (b)= no. of experimental revertant colony forming units: (c)=no of assayed spontaneous revertants: (d)= no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$: act = activation with S-9

Table-6-A

Salmonella/Microsome Assay

| Substa | nce Assayed:_ | Code #37 | | Dissolved | In: | DMS0 |
|--------|---------------|--------------|-------------|-----------|-------|-------------|
| Date: | 12 Nov 80 | Performed By | : Kincannon | . Sauers | Summe | rs. Pulliam |

| Test Compound and Concentration | (a) | (b) | E act | (c) | C act | E-C (d) | E-C act | CAV (e) | C _{AV} act | MUTAR (f) | MUTA act |
|---------------------------------------|---------|----------|----------|-----|----------|------------|------------|------------|------------------------|--------------|-------------|
| 1.0 mg/pl | TA 1533 | 5 | 7 | 10 | 14 | | | w | | | |
| 0.2 mg/p1 | TA 1538 | 10 | 21 | 10 | 14 | | 7 | | 17.1 | | 0.41 |
| 0.04 mg/p1 | TA 1538 | 10 | 25 | 10 | 14 | | 11 | | 17.1 | | 0.64 |
| 0.008 mg/pl | TA 1538 | 9 | 17 | 10 | 14 | | 3 | | 17,1 | | 0.17 |
| 0.0016 mg/p1 | TA 1538 | 12 | 14 | 10 | 14 | 2 | | 8.3 | | 0,24 | |
| 0.00032 mg/p1 | TA 1538 | 5 | 13 | 10 | 14 | | | | <u></u> | | |
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⁽a)- tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) - E-C/ C_{AV} act = activation with S-9

Table-6-B

MUTAGENIC ACTIVITY RATIO WORKSHEET Salmonella/Microsome Assay

Substance Assayed: Code #73A Dissolved In: DMSO Date: 12 Nov 80 Performed By: Pulliam, Sauers

| Test Compound and Concentration | TA (a) | (b) | E act | (c) | C act | (d) | E-C act | CAV (e) | C _A y act | | 37. |
|---------------------------------------|-----------|----------|----------|----------|----------|-------------------------------|------------|------------|-------------------------|----------|--------------|
| 1.0 mg/pl | TA 98 | 9 | 14 | 22 | 26 | | | | | | |
| 0.2 mg/p1 | TA 98 | 14 | 21 | 22 | 26 | | | | | | |
| 0.04 mg/pl | TA 98 | 16 | 21 | 22 | 26 | | | | · | : | |
| 0.008 mg/pl | TA 98 | 14 | 16 | 22 | 26 | | | | | | - - |
| 0.0016 mg/pl | TA 98 | 12 | 24 | 22 | 26 | ····· 114 41 44 44 | | | | | with the ray |
| 0.00032 mg/pl | TA 98 | 13 | 15 | 22 | 26 | | | | | | |
| | | | | <u> </u> | | | | | | | _ |
| 1.0 mg/pl | TA 100 | 47 | 54 | 115 | 112 | | | | | - | |
| 0.2 mg/p1 | TA 100 | 63 | 68 | 115 | 112 | | | | | | - |
| 0.04 mg/pl | TA 100 | 80 | 90 | 115 | 112 | | | | | <u> </u> | - |
| 0,008 mg/p1 | TA 100 | 65 | 90 | 115 | 112 | | | | · | ; | |
| 0,0016 mg/pl | TA 100 | 74 | 95_ | 115 | 112 | | | | | : | _ |
| 0.00032 mg/p1 | TA 100 | 76 | 80_ | 115 | 112 | | | | | , | |
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⁽a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)-no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from nustorical data. (f) = $E-C/C_{AV}$: act = activation with >-9

Table-6-B

Salmonella/Microsome Assay

Substance Assayed: Code #73A Dissolved In: DMSO

| Date | e: <u>12 No</u> | <u>08 v</u> | Per | formed | Ву: _ | Pullia | m, Sauer | 5 | . ———. — | |
|---|---|------------------------|------------------|----------------------|----------------------|------------|------------|------------|-------------------------|-----------------------|
| Test Compound and Concentration | TA (a) | (b) | E act | (c) | Cact | E-C (d) | E-C act | CAy (e) | C _A v act | MUTAR MUTA (f) act |
| 1.0 mg/pl | TA 1535 | 9 | 5 | 13 | 5 | | | | | , |
| 0.2 mg/pl | TA 1535 | 8 | 7 | 13 | 5 | | 2 | | 13.3 | 0.15 |
| 0.04 mg/p1 | TA 1535 | 10 | 7 | 13 | 5 | | 2 | | 13.3 | 0.15 |
| Q.008 mg/pl | TA 1535 | 7 | 8 | _13 | 5 | | 3 | | 13.3 | 0.45 |
| 0.0016 mg/p1 | TA 1535 | 7 | 8 | 13 | 5 | | 3 | | 13.3 | 1 0.45 |
| 0.00032 mg/p1 | TA 1535 | 5 | 9 | 13_ | 5 | | 4 | | 13.3 | 0.61 |
| 1.0 mg/pl | TA 1537 | 4 | 2 | 11 | 10 | | | | | , |
| 0.2 mg/pl | TA 1537 | 5 | 6 | 11_ | 10 | | | | | |
| 0.04 mg/pl | TA 1537 | 8 | 6 | 1.11. | 10 | | | | | <u>'</u> |
| 0.008 mg/p) | TA 1537 | 10 | 6 | 11_ | 10 | | | | | |
| 0.0016 mg/pl | TA 1537 | 7 | 9 | <u></u> | 10 | | | | | |
| 0.00032 mg/p) | TA 1537 | _5 | 7 | 11_ | 10_ | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | 1 |
| 1.0 mg/p] 0.2 mg/p] 0.34 mg/p] 0.008 mg/p] 0.0016 mg/p] | TA 1537 TA 1537 TA 1537 TA 1537 TA 1537 | 4 5 8 10 7 | 2 6 6 6 | 11 11 11 11 | 10 10 10 10 | | 4 | | 13.3 | |

(a)- tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of as ayed spontaneous revertants: (d)=no, revertants in excess of the assazed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data (f) = E-C/C_{AV}: act = activation with S-9

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Table-6-B

Substance Assayed: Code #73A

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Dissolved In: DMSO

| Dat | e: <u>12 Nov</u> | 80 | Per | formed | Ву: _ | Sauer | <u>s</u> | | | | |
|---------------------------------------|------------------|----------|----------|--------|-------|------------|------------|-------------|------------------------|--------------|-------------|
| Test Compound and Concentration | (a) | E (b) | E act | (c) | Cact | E-C (d) | E-C act | CAV (e) | C _{AV} act | HUTAR (f) | MU-, act |
| 1.0 mg/pl | TA 1538 | 8 | 15 | 10 | 14 | | 1 | | 17.1 | | 0.06 |
| 0.2 mg/pl | TA 1538 | 10 | 14 | 10 | 14 | | | | | | |
| 0.04 mg/pl | TA 1538 | 8 | 22 | 10 | 14 | | 6 | | 17.1 | | 0.35 |
| 0.008 mg/pl | TA 1538 | 6 | 12 | 10 | 14 | | | | | | |
| 0.0016 mg/pl | TA 1538 | 10 | 14 | 10 | 14 | | | - | | | |
| 0.00032 mg/p1 | TA 1538 | 8 | 14 | 10 | 14 | | | | | | |
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(a)= tester strain: (b)=no, of experimental revertant colony forming units: (c)=no, of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from nistorical data: (f) = $E-C/C_{AV}$: act = activation with S-3

Table-6-C MUTAGENIC ACTIVITY RATIO WORKSHEET

| Subs | tance As: | sayed: | Cod | e #83 | | | Dissolv | ved In: _ | DMS |) | |
|--|-----------|--------|----------|--------|------|------------|------------|------------|------------------------|---------------|-------------|
| Date | : 18 N | ov 80 | Per | formed | By: | Pulliam, | Sauers | ···· | | | |
| - | | | | | | | - | | | | - |
| Test Compound and Concentration | (a) | (b) | E act | (c) | act | E-C (d) | E-C act | CAV (e) | C _{AV} act | HUTAR: (f) | MUTA |
| 1.0 mg/p] | TA 98 | 11_ | 10 | 2 | 36 | 9 | | 23.1 | | 0.39 | |
| 0.2 mg/pl | TA 98 | 19_ | 22 | 2 | 36 | 17. | | 23.1 | | 0.74 | |
| 0.04 mg/pl | TA 8 | _17_ | 19 | 2 | 36 | 15 | - | 23.1 | | 0.65 | |
| | TA SA | . 19 | 22 | 2 | 36 | 17 | | 23.1 | | 0.74 | |
| 0.0016_mg/p1 | TA 98 | 20 | 17 | 2 | 36 | 18 | | 23.1 | | 0.78 | |
| 0.00032 mg/pl | TA 98 | _12_ | 15 | 2 | 36 | _10 | | 23.1 | | 0.43 | |
| | | | | | | | | | | <u> </u> | |
| | TA 100 | 47 | 40 | | 1.30 | | | 100 | | 0.05 | - |
| 0.2 mg/pl | TA 100 | _81 | | | 130 | 5 | | 106 | | 0.05 | |
| 1 | TA 100 | 84 | 96 | | 130 | 8 | | 196 | | 0.07 | |
| 0.008_mg/pl | TA_100_ | _85 | .84 | 76 | 130 | 9 | | 196 | | 0.98 | |
| 0.0016_mg/pl | TA_100 | 66 | 60 | 76 | 130 | | | | | | |
| 0.00032_mg/pl | TA_:00_ | 5) | 83 | 76 | 30 | | | | | | |
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| Salar St. Str. Market St. Strate St. 1975, again | | | | | | | | | | | |
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(a)= tester strain: (b)=no, of experimental revertant colony forming units: (c)=no, of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) - $E=C_{V}C_{AV}$: act - activation with S-9

Table-6-C

Salmonella/Microsome Assay

Substance Assayed: Code #83 Dissolved In: DMSO

| Date | e: <u>18 No</u> | 08 vo | Per | formed | Ву: _ | Pulliam, | Sauers | | | ************************************** | |
|---------------------------------------|-----------------|-------|----------|--------|----------|------------|------------|-------------|------|--|-------------|
| Test Compound and Concentration | TA (a) | (b) | E act | (c) | C act | E-C (d) | E-C act | CAV (e) | CAV | MUTAR (f) | MUT: act |
| 1.0 mg/p1 | TA 1535 | 4 | 6 | 5 | 6 | | | | | | |
| 0.2 mg/pl | Ta 1535 | 9 | 7 | 5 | 6 | 4 | 1 | 9.4 | 13.3 | 0.43 | 0.03 |
| 0.04 mg/pl | TA 1535 | 5 | 7 | 5 | 6 | | | | 13.3 | | 0.08 |
| 0.008 mg/p1 | TA 1535 | 6 | 6 | 5 | 6 | J | | 9.4 | | 0.11 | |
| 0.0016 mg/p1 | TA 1535 | 6 | 4 | 5 | 6 | 1 | | 9.4 | | 0.11 | |
| 0.00032 mg/p1 | TA 1535 | 6 | 7 | 5 | 6 | 1 | 1 | 9.4 | 13.3 | 0.11 | 0.08 |
| 1.0 mg/pl | TA 1537 | 1 | 1 | 3 | 5 | | | | | | |
| 0.2 mg/pl | TA 1537 | 3 | 4 | 3 | 5 | | | | ļ | | |
| 0.04 mg/p1 | TA 1537 | 5 | 3 | 3 | 5 | 2 | | 6.1 | ļ | 0.33 | |
| 0.008 mg/pl | TA 1537 | 3 | 3 | 3 | 5 | | | · | | | |
| 0.0016 mg/p1 | TA 1537 | 1 1 | 4 | 3 | 5 | | | | | | |
| 0.00032 mg/pl | TA 1537 | 3 | 2 | 3 | 5 | | | | | | |
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(a)= tester strain: (b)=no, of experimental revertant colony forming units: (c)=no, of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from hi torical data. (f) = $E-C/C_{AV}$: act = activation with 5-9

Table 6-C

Substance Assayed: Code #83

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

___ Dissolved In: __DMSO

| Date | : 18 1 | lov 80 | Per | formed | Ву: _ | Sauer | <u>`s</u> | | | | |
|---------------------------------------|-----------|----------|----------|----------|-------|------------|------------|------------|------|--------------|-------------|
| Test Compound and Concentration | †A (a) | E (b) | E act | (c) | Cact | E-C (d) | E-C act | CAV (e) | CAV | HUTAR (f) | MUTA act |
| 1.0 mg/pl | TA 1538 | 4 | 7 | 2 | 11 | 2 | | 8.3 | | 0.24 | |
| 0.2 mg/pl | TA 1538 | 3 | 12 | 2 | 11 | ١ | 1 | 8.3 | 17.1 | 0.12 | 0.06 |
| 0.0% mg/pl | TA 1538 | G | 12 | 2 | 11 | 4 | 1 | 8.3 | 17.1 | 0,48 | 0.06 |
| 0.008 mg/p1 | TA 1538 | 7 | 15 | 2 | 11 | 5 | 4 | 8.3 | 17.1 | 0.61 | 0.23 |
| 0.0016 mg/p1 | TA 1538 | 13 | 7 | 2 | 11 | 11 | | 8.3 | | 1.32 | |
| 0.00032 mg/p | 1 TA 153 | 8 6 | 11 | 2 | 11 | 4 | | 8.3 | | 0.48 | |
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| | 1 | 1 | 1 | | 1 | | | | 1 | 1 | 1 |

(a)= tester strain. (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontuneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C, C_{AV} : act = activation with S-9

Table-6-D

Salmonella/Microsome Assay

| Substance Assayed: | Code #53 | Dissolved In | 1: DMSO |
|--------------------|------------------|---------------|---------|
| Date: 18 Nov 80 | Performed By: Pu | lliam, Sauers | |

| Test Compound and Concentration | TA (a) | (b) | E act | (c) | C act | E-C (d) | E-C act | CAV (e) | C _A y act | HUTAR (f) | ATUM act |
|---------------------------------------|------------------|----------|----------|-----|----------|------------|------------|------------|---------------------------------------|--------------|-------------|
| 1.0 mg/pl | TA 98 | 17 | 25 | 2 | 36 | 15 | | 23.1 | · · · · · · · · · · · · · · · · · · · | 0.65 | |
| 0.2 mg/pl | TA 98 | 15 | 20 | 2 | 36 | 13 | | 23.1 | | 0.56 | |
| 0.04 mg/pl | TA 98 | 17 | 18 | 2 | 36 | 15 | | 23.1 | | 0.65 | |
| 0.008 mg/pl | TA 98 | 17 | 23 | 2 | 36 | 15 | | 23.1 | | 0.65 | |
| 0.0016 mg/pl | TA 98 | 16 | 25 | 2 | 36 | 14 | | 23.1 | | 0.60 | |
| 0.00032 mg/pl | TA 98 | 13 | 25 | 2 | 36 | 11 | | 23.1 | | 0.48 | |
| 1.0 mg/pl | TA 100 | 66 | 74 | 76 | 130 | | | | | | |
| 0.2 mg/pl | TA 100 | 89 | 86 | 76 | 130 | 13 | | 106 | | 0.12 | |
| 0.04 mg/pl | TA 100 | 75 | 85 | 76 | 130 | | | | | | |
| 0.008 mg/pl | TA 100 | 84 | 93 | 76 | 130 | 88 | | 106 | | 0.08 | |
| 0.0016 mg/pl | TA 100 | 87 | 93 | 76 | 130 | 11 | | 106 | | 0.10 | |
| 0.00032 mg/pl | TA 100 | 70 | 90 | 76 | 130 | | | | | | |
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/C_{AV}: act = activation with S-9

Table-6-D MUTAGENIC ACTIVITY RATIO WORKSHEET

Substance Assayed: Code #53 Dissolved In: DMSO

| Date |): - | 18 No | v 80 | Per | formed | Ву: _ | <u>Pulliam,</u> | Sauers | <u></u> | | | |
|---------------------------------------|---------|-----------|------|----------|--------|----------|-----------------|------------|------------|-------------------------|--------------|-------------|
| Test Compound and Concentration | | `A (a) | (p) | E act | (c) | C act | E-C (d) | E-C act | CAV (e) | C _A y act | MUTAR (f) | MUTA act |
| 1.0 mg/pl | TA | 1535 | 6 | 4 | 5 | 6 | | | 9,4 | 13.3 | 0.11 | |
| 0.2 mg/pl | TA | 1535 | 8 | 7 | 5 | 6 | 3 | 1 | 9.4 | 13,3 | 0.32 | 0.08 |
| 0.04 mg/p] | TA | 1535 | 9 | 9 | 5 | 6 | 4 | 3 | 9.4 | 13.3 | 0.43 | 0.23 |
| 1a\pm_800.0 | TA | `535 | 6 | 9 | 5 | ű | 7 | 7 | 9.4 | 13.3 | 0.76 | 0.08 |
| 0.0016 mg/p1 | TA | 1535 | 6 | 9 | 5 | 6 | 1 | 3 | 9.4 | 13.3 | 0.11 | 0.23 |
| 0,00032 mg/p1 | TA | 1535 | 13 | 8 | 5 | 6 | 8 | 2 | 9,4 | 13.3 | 0.89 | 0.15 |

TA 1537

TA 1537

TA 1537

1.0 mg/pl

 $0.2 \, \text{mg/pl}$

0.04 mg/pl

| 0.008 mg/pl | TA 1537 | 6 | 5 | 3 | _5 | 3 | | 6.1 | | 0.49 | |
|---------------|--------------|----------------------|---|--------------------------|----------------------------|------------------------------|--------------------------------|----------------------------------|--------------------------------------|--|---|
| 0.0016 Jng/pl | TA 1537 | 9 | 6 | 3 | 5 | 6 | | 6.1 | 7.5 | 0.98 | 0.13 |
| | ì | ١, | 6 | 3 | 5 | | 1 | | 7.5 | | 0.13 |
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| | | | | | | | | | | | |
| | 0.0016 mg/pl | 0.0016 mg/pl TA 1537 | 0.008 mg/pl TA 1537 6 0.0016 Jng/pl TA 1537 9 0.00032 mg/pl TA 1537 3 | 0.0016 mg/pl TA 1537 9 6 | 0.0016 mg/pl TA 1537 9 6 3 | 0.0016 mg/p1 TA 1537 9 6 3 5 | 0.0016 mg/pl TA 1537 9 6 3 5 6 | 0.0016 mg/pl TA 1537 9 6 3 5 6 1 | 0.0016 mg/p1 TA 1537 9 6 3 5 6 1 6.1 | 0.0016 mg/p1 TA 1537 9 6 3 5 6 1 6.1 7.5 | 0.0016 mg/p1 TA 1537 9 6 3 5 6 1 6.1 7.5 0.98 |

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0.16

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$ act = activation with S-9

Table-6-D MUTAGENIC ACTIVITY RATIO WORKSHEET

| Sub | stance As | sayeo: | <u> </u> | ode #5 | 3 | | _ D12201/ | /ea in: - | บกรบ | | |
|---------------------------------------|---------------------|----------|----------|----------|-------|------------|------------|------------------------|------------------------|--------------|--------------|
| Date | e: <u>18 No</u> | 08 v | Per | formed | Ву: _ | Pu]] 1 | iam, Saue | ers | | - | |
| Test Compound and Concentration | (a) | (b) | E act | (c) | Cact | E-C (d) | E-C act | C _{AV} (e) | C _{AV} act | HUTAR (f) | MUT: act |
| 1.0 mg/pl | TA 1538 | 14 | 13 | 2 | 11 | 12 | 2 | 8.3 | 17.1 | 1.45 | 0.13 |
| 0.2 mg/pl | TA 1538 | 9 | 9 | 2 | 11 | 7 | | 8.3 | | 0.84 | <u> </u> |
| 0.04 mg/p1 | TA 1538 | 5 | 11 | 2 | 11 | 3 | | 8.3 | | 0.36 | |
| 0.008 mg/pl | TA 1538 | 111 | 16 | 2 | 11 | 9 | 5 | 8.3 | 17.1 | 1.08 | 0.24 |
| 0.0016 mg/pl | TA 1538 | 6 | 16 | 2 | 11 | 4 | 5 | 8.3 | 17.1 | 0.48 | 0.29 |
| 0.00032 mg/p1 | TA 1538 | 9 | 11 | 2 | 11 | 7 | ļ | 8.3 | ļ | 0.84 | |
| | ļ | <u> </u> | <u> </u> | ļ | | | | | | | |
| | ļ | <u> </u> | | | | | ļ | | | | <u> </u> |
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$: act = activation with S-9

Table 6-E
MUTAGENIC ACTIVITY RATIO WORKSHEET

| Substance Assayed: <u>Code #91</u> | Dissolved In: DMSO |
|------------------------------------|--------------------|
| Date: 18 Nov. 80 Performed By: | Pulliam, Sauers |

| Test Compound and Concentration | Ţλ (a) | £ (b) | E act | (c) | C act | (d) | E-C act | CAV (e) | C _{AV} act | MUTAR: | MUTA act |
|---------------------------------------|-----------------------|----------|----------|-----|----------|-----|------------|------------|------------------------|--------|-------------|
| 0.01 mg/plate | TA 98 | 19 | 21 | 2_ | 36 | 17 | | 23.1 | | 0.74 | |
| 2 x 10 ⁻³ | TA 98 | 20 | 21_ | 2_ | 36 | 18 | | 23,1 | | 0.78 | |
| 4 x 10 ⁻⁴ | TA 98 | 12_ | 23 | _2_ | 36 | 10 | | 23,1 | ~~~· | 0,43 | |
| 8 x 10 ⁻⁵ | TA 98 | _15 | 24 | _2_ | 36_ | 13 | | 23,1 | | 0,56 | |
| 3.2 × 10 ⁻⁶ | TA 98 | _22 | 19 | _2_ | 36 | 20 | | 23,1 | | 0.87 | |
| 0.01 mg/plate | TA 100 | 73 | .77 | 76 | 130 | | | | | | |
| 2 x 10 ⁻³ | TA_100 | 66 | 69 | 76_ | 130 | | | | | | |
| 6 × 10-4 | TA_100 | | . 75 | 76. | 130 | | | | | | |
| 8 x 10-5 | TA 100 | 36 | 76 | 76 | 130 | | | | | | , |
| 3.2 x 10 ⁻⁶ | TA 100 | 84_ | _77 | 76 | 130 | | | | | | |
| 0.01 mg/plate | TA 1535 | 9 | 9 | 5 | . 6. | 4 | 3 | 9.4 | 13.3 | 0.43 | 0.2 |
| 2 x 10 ⁻³ | TA 1535 | 6_ | 7 | 5 | 6 _ | | 1_ | 9.4 | 13.3. | 0.11 | 0.0 |
| 4 x 10 ⁻⁴ | TA 1535 | 9 | 7 | 5 | 6 | 4 | 11 | _9.4 | 13.3 | 0.43 | _0.0 |
| 8 x 10 ⁻⁵ | <u>TA_ 1535</u> | 6 | 10_ | 5_ | 6 | 1 | 4_ | 9.4 | 13.3 | 0.11 | _0.3 |
| 3.2 × 10 ⁻⁶ | TA 1535 | _10. | 9 | 5_ | 6_ | 5 | 3 | 9.4. | 13.3 | 0.53 | 2.2 |

⁽a)= tester strain: (b)=no, of experimental revertant colony forming units: (c)=no, of assayed spontaneous revertants: (d)=no, revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$ act = activation with S-9

TAble 6-E
MUTAGENIC ACTIVITY RATIO WORKSHEET

| Substance Assayed: | Code # 91 | Dissolved I | in: <u>DMSO</u> |
|------------------------|---------------|-----------------|-----------------|
| Date: <u>18 Nov 80</u> | Performed By: | Pulliam, Sauers | |

| Test Compound | TA (a) | (b) | E | (c) | C | E-C (d) | E-C act | CAV (e) | CAV | HUTAR (f) | MUT - |
|------------------------|----------|-----|------------|-----|------|------------|------------|------------|------|--------------|-------|
| Concentration | (4) | | | | 400 | (0) | | (6) | | \',' | ! |
| 0.01 mg/plate | TA 1537 | 3 | 4 | 3 | 5 | | | | | | · |
| 2 x 10 ⁻³ | TA 1537 | 4 | 3 | 3 | 5 | 1 | | 6.1 | | 0.16 | ! |
| 4 × 10 ⁻⁴ | TA 1537 | 6 | 4 | 3 | 5 | 3 | | 6.1 | | 0,49 | ì |
| 8 x 10 ⁻⁵ | TA 1537 | 3 | 6_ | 3 | 5 | | 111 | | 7.5 | | C.13 |
| 3.2 × 10-6 | ግለ 1537 | 4 | 7 | 3 | 5 | 11 | 2 | 6.1 | 7.5 | 0,16 | 1.2. |
| | | | | | | | | | | | İ |
| 0.01 mg/plate | TA 1538 | 8 | 10 | 2 | _11_ | .6 | | 8.3 | | 0.73 | |
| 2 × 10 ⁻³ | TA 1538 | 6 | 8 | 2_ | 11 | 4 | | 8.3 | | 0.48 | ! |
| 4 × 10 ⁻⁴ | T.\ 1530 | 6 | 9 | 2 | 11 | 4 | | 8.3 | | 0.48 | 1 |
| 8 x 10 ⁻⁵ | TA 1538 | 6 | 11 | 2 | 11 | 4 | | 8.3 | | 0,48 | 1 |
| 3.2 x 10 ⁻⁶ | TA 1538 | 9 | 15 | 2 | 11 | 7 | 4 | 8.3 | 17.1 | 0.84 | 0.23 |
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⁽a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=nc. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$: act = activation with S-9

Table-6-F
MUTAGENIC ACTIVITY RATIO WORKSHEET
Salmonella/Microsome Assay

| Subs | stance As | sayed: | Cod | e # 37 | | | Dissolv | ed In: j | DMSO | | |
|---|-----------------|----------|----------|--------|----------|------------|------------|----------------|------------------------|--------------|------------|
| Date | e: <u>11 De</u> | c 80 | Per | formed | Ву: _ | Pulli | am. Saue | rs | · | | |
| Test Compound and Concentration | (a) | E (b) | E act | (c) | C ac+ | E-C (d) | E-C act | CAV (e) | C _{AV} act | HUTAR (f) | MUT/ ac |
| 1.0 mg/p1 | TA 1537 | 3 | 6 | 7 | 8 | | | - | | | |
| 0.2 mg/p1 | TA 1537 | 4 | 4 | 7 | 8 | | | | | | <u> </u> |
| 0.04 mg/pl | TA 1537 | 5 | 9 | 7 | 8 | | 11 | | 7.5 | | 0.13 |
| 0.008 mg/pl | TA 1537 | 4 | 7 | 7 | 8 | | | | | | |
| 0.0016 mg/pl | TA 1537 | 7 | 7 | 7 | 8 | | | | <u></u> | | |
| 0.90032 mg/pl | TA 1537 | 4 | 4 | 7 | 8 | | | | | | |
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$: act = activation with S-9

Table-6-G

Substance Assayed: Code #53

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

__ Dissolved In: <u>DMSO</u>

| Date | e: <u>11 De</u> | c 80 | Per | formed | By: _ | Pullia | ım, Sauer | 's | | | |
|---------------------------------------|-----------------|--------------|----------|--------------|----------------|--------------|--------------|---------------|------------------------|--------------|----------|
| Test Compound and Concentration | (a) | E (b) | E act | (c) | C | E-C (d) | E-C act | CAV (e) | C _{AV} act | HUTAR (f) | MUT; |
| | TA 1537 | 6 | 6 | 7 | 8 | | | | | | |
| 0.2 mg/pl | TA 1537 | 5 | 7 | 7 | 8 | | | | | | |
| 0.04 mg/pl | TA 1537 | 4 | 7 | 7 | 8 | | | | <u> </u> | | |
| 0.008 mg/pl | TA 1537 | 4 | 10 | 7 | 8 | | 2 | L | 7.5 | | 0.20 |
| 0.0016 mg/pl | TA 1537 | 4 | 7 | 7 | 8 | | | | | | |
| 0.00032 mg/pl | TA 1537 | 6 | 6 | 7 | 8 | | | | | | |
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data. (f) = $E-C/C_{AV}$: act = activation with S-9

Table-6-H

Substance Assayed: Code #83

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Dissolved In: __DMSO

| Date | e: <u>11 De</u> | c 80 | Per | formed | By: _ | Pulliam | , Sauers | | | | |
|---------------------------------------|-----------------|------|----------|--------|-------|------------|------------|---------------------------------------|-------------------------|--------------|-------------|
| Test Compound and Concentration | (a) | (b) | E act | (c) | Cact | E-C (d) | E-C act | CAV (e) | C _A y act | MUTAR (f) | MUTA act |
| 1.0 mg/pl | TA 1537 | 6 | 4 | 7.3 | 8 | | | | | | · |
| 0.2 mg/pl | TA 1537 | 4 | 6 | 7.3 | 3 | | | | | | |
| 0.04 mg/pl | TA 1537 | 4 | 7 | 7.3 | 8 | | | | | | |
| 0.008 mg/pl | TA 1537 | 7 | 8 | 7.3 | 8 | | | · · · · · · · · · · · · · · · · · · · | | | |
| 0.0016 mg/pl | TA 1537 | 5 | 5 | 7.3 | 8 | | | | | | |
| 0.00032 mg/pl | TA 1537 | 7 | 7 | 7.3 | 8 | | | | | | |
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$: act = activation with S-9

Table 6-I MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

| Subs | stance As | sayed: | Co | de #91 | | | Dissolv | ed In: | DMSO | | |
|---------------------------------------|--------------|----------|----------|----------------|----------------|------------|------------|---------------------------------------|--------------|--------------|--|
| Date | :: <u> </u> | ec 80 | Per | formed | Ву: _ | Sauers. | Fulliam | | - | | |
| Test Compound and Concentration | (a) | E (b) | E act | (c) | Cact | E-C (d) | E-C act | CAY (e) | CAV | HUTAR (f) | MUTAI act |
| 0.01 mg/plat | TA 153 | 5_ | | 7 | 8 | | | · · · · · · · · · · · · · · · · · · · | | | |
| 2 x 10-3 | TA 153 | 6_ | 9 | 7 | 8 | | 1 | | 7.5 | | 0.13. |
| 4 × 10-4 | TA 153 | 5_ | 6_ | | 8_ | | | | | | <u> </u> |
| 8 x 10-5 | TA 1537 | . 7 | 8_ | | 8 | | | | | | |
| 1.6 × 10-5 | TA 1537 | 7 | 6_ | | 8 | | | | | | |
| 3.2 x 10 ⁻⁶ | TA 1537 | 5 | 7 | | 8_ | | | | | | |
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$: act = activation with S-9

Table-6-J

Salmonella/Microsome Assay

| Subs | stance As | sayed: | Cod | e #73A | | · · · · · · · · · · · · · · · · · · · | _ Dissolv | ed In: _ | DMSO | | |
|---------------------------------------|--|--------------|--------------|--------------|----------------|---------------------------------------|------------|--------------|------------------------|--------------|-------------|
| Date | : <u>11 De</u> | c 80 | Per | formed | Ву: _ | Pullia | am. Sauer | 'S | | | |
| Test Compound and Concentration | (a) | E (b) | E act | (c) | C act | E-C (d) | E-C act | CAV .! | C _{AV} act | MUTAR (f) | MUTAR |
| 1.0 mg/pl | TA 1537 | 7 | 6 | 7.3 | 8 | | | | | | |
| 0.2 mg/pl | TA 1537 | 6 | 5 | 7.3 | 8 | | | | | | |
| 0.04 mg/pl | TA 1537 | 4 | 6 | 7.3 | 8 | | | | | | |
| Q.QQ8_mg/pl | TA 1537 | 4 | 9 | 7.3 | 8 | | 1 | | 7.5 | | 0.13 |
| 0.0016 mg/pl | TA 1537 | 6 | 7 | 7.3 | 8 | | | | | | |
| 0.00032 mg/p1 | TA 1537 | 4 | 7 | 7.3 | 8 | | | | | | |
| | | | | | | | | | | | |
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/ \cup AV: act = activation with S-9

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Table-6-K

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

| Substa | ince Assayed: | Code #53 | Dissolved In: DMSO |
|--------|---------------|---------------|--------------------|
| Date: | 16 Dec 80 | Performed By: | Pulliam, Sauers |

| Test Compound and Concentration | TA (a) | (p) | E act | (c) | C act | E-C (d) | E-C act | C _A y (e) | CAV | fiutar (f) | MUTAR |
|---------------------------------------|-------------------|-----|----------|-----|----------|------------|------------|-------------------------|---------------------------------------|---------------|-------|
| 1.0 mg/pl | TA 98 | 14 | 18 | 25 | 30 | | | | | | |
| 0.2 mg/pl | TA 98 | 16 | 17 | 25 | 30 | | | | · · · · · · · · · · · · · · · · · · · | | |
| 0.04 mg/pl | TA 98 | 19 | 17 | 25 | 30 | | | | | | |
| 0.008 mg/pl | TA 98 | 15 | 10 | 25 | 30 | | | | | | |
| 0.0016 mg/pl | TA 98 | 17 | 20 | 25 | 30 | | | | | | |
| 0.00032 mg/pl | TA 98 | 11 | 8 | 25 | 30 | | | | | | |
| 1.0 mg/p1 | TA 1538 | 9 | | 15 | 18 | | | | | | |
| 0.2 mg/pl | TA 1538 | 14 | | 15 | 18 | | | | | | |
| 0.04 mg/pl | TA 1538 | 10 | | 15 | 18 | | | | | | |
| 0.008 mg/pl | TA 1538 | 7_ | | 15 | 18 | | | | | | |
| 0.0016 mg/p1 | TA 1538 | 9 | ! ! | 15 | 18 | | | | | | |
| 0.00032 mg/pl | TA 1538 | 7 | | 15 | 18 | | | | | | |
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⁽a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$: act = activation with S-9

Table-6-L

Salmonella/Microsome Assay

| Substa | ance | Assayed:_ | Code #83 | | | Dissolved | In: | DMSO |
|--------|------|-----------|-----------|-----|----------|-----------|-----|------|
| Date: | 16 | Dec 80 | Performed | By: | Pulliam, | Sauers | | |

| Test Compound and Concentration | TA (a) | (b) | E act | (c) | Cact | E-C (d) | E-C act | CAY (e) | C _A y act | MUTAR (f) | MUTAR act |
|---------------------------------------|-----------|--------------|----------|----------|------|------------|------------|------------|-------------------------|--------------|--------------|
| 1.0 mg/pl | TA 98 | 9 | 16 | 25 | 30 | | | | | | |
| 0.2 mg/pl | TA 93 | 11 | 17 | 25 | 30 | | | | | | |
| 0.04 mg/pl | TA 98 | 16 | 19 | 25 | 30 | | | | | | |
| 0.008 mg/pl | TA 98 | 14 | 21 | 25 | 30 | | | | | | |
| 0.0016 mg/pl | TA 98 | 17 | 20 | 25 | 30 | | | | | | |
| 0.00032 mg/pl | TA 98 | 17 | 16 | 25 | 30 | | | | | | |
| 1.0 mg/pl | TA 1538 | 10 | | 15 | 18 | | | | | | |
| 0.2 mg/pl | TA 1538 | 11 | | 15 | 18 | | | | | | |
| 0.04 mg/pl | TA 1538 | 14 | | 15 | 18 | | | | | | |
| 0.008 mg/p1 | TA 1538 | 8 | | 15 | 18 | | | | | | |
| 0.00032 mg/pl | TA 1538 | 6 | | 15 | 18 | · | | | | | |
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(a)= tester frain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = $E-C/C_{AV}$: act = activation with S-9

Table-6-M

Salmonella/Microsome Assay

| Subs | Code #91 | | | | _ Dissolv | ed In: | DMSO | | | | |
|---|----------------|-------|----------|--------|-----------|------------|------------|------------|----------|--------------|--------------|
| Date | : <u>16 De</u> | ec 80 | Per | formed | By: _ | Pulliam | , Sauers | | | | |
| Test Compound and Concentration | TA (a) | (b) | ë act | (c) | C act | E-C (d) | E-C act | CAV (e) | Сду | HUTAR (f) | MUT: act |
| 0.001 | TA 98 | 8_ | 19 | 25 | 30 | | | | | | |
| 0.002 mg/ml | TA 98 | 14 | 22 | 25 | 30 | | | | | | |
| 0.0004 mg/pl | TA 98 | 11_ | 18 | 25 | 30 | | | | | | |
| 0.00008mg/pl | TA 98 | 13 | 19 | 25 | 30 | | | | | | |
| 0.000016mg/p1 | TA 98 | 14 | 23 | 25 | 30 | | | | | | |
| 0.0000032mg/pl | TA 98 | 13 | 14 | 25 | 30 | | | | | | |
| D.01 mg/pl | TA 1538 | 6 | | 15 | 18 | | | | | | |
| 0.002 mg/p1 | TA 1538 | 10 | | 15 | 18 | | | | | | |
| 0.0004 mg/pl | TA 1538 | 12 | | 15 | 18 | | | | <u> </u> | | |
| 0.00008 mg/pl | TA 1538 | 10 | <u> </u> | 15 | 18 | | | | | | |
| 1 1 1 1 1 2 1 2 1 1 1 1 1 1 1 1 1 1 1 1 | TA 1538 | 14 | | 15 | 19 | | | | | | |
| D.0000032mg/pl | TA 1538 | 8 | <u></u> | 15 | 18 | | | | <u> </u> | | |
| | ! ! | | <u></u> | | | | | | | <u> </u> | |
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(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data (f) $E-C/C_{\rm AV}$: act = activation with S-9

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